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Original Article

Role of aminoguanidine and nicorandil in oxidative stress in streptozotocin- induced diabetes mellitus in rats

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A B S T R A C T

Abstract: The effect of aminoguanidine, nicorandil and their combination against oxidative stress in streptozotocin (STZ) - induced diabetes mellitus was assessed in rats by determining changes in blood glucose level, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), malondialdehyde (MDA) and nitric oxide (NO) in healthy and experimentally induced diabetic rats. Besides, histhopathological examination of kidney and liver tissues was performed. Diabetic rats were randomized into groups of six rats and received 50mg/kg, intraperitoneally of aminoguanidine (an anti- advanced glycation end products (AGE) which prevents the formation of reactive oxygen species and lipid peroxidation in cells), 0.1mg/kg, orally of nicorandil (nicotinamide derivative which is efficacious in the treatment of hypertension and angina pectoris and a potassium channel opener) and their combination once daily for one month. Blood glucose level was significantly elevated in plasma of diabetic rats. SOD, CAT and GSH levels were significantly reduced, while MDA and NO levels were significantly elevated in plasma of diabetic rats. Abnormalities in both kidney and liver structures of diabetic rats were observed. Treatments of the diabetic rats with aminoguanidine, nicorandil and their combination led to improvement of the abnormalities in SOD, CAT, GSH, MDA, NO and also the histhopathological abnormalities of kidney and liver. From these results it can be concluded that aminoguanidine, nicorandil and their combination have the ability to attenuate oxidative stress induced by streptozotocin. This effect is positively correlated with their anti-oxidant activities.

Key Words: Aminoguanidine, Nicorandil, Oxidative stress, Streptozotocin, Diabetes, Histopathology

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1. INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both (Kumar and Clark, 2002). Since diabetes mellitus is a heterogeneous disorder with multiple causes, the beneficial effects of combined therapeutic agents aiming at specific pathobiological pathways of diabetes and its complications have been reported (Tiwari and Madhusudana-Rao, 2002 and Sharma and Srinivasan, 2009).

The pharmacological agents currently employed, such as sulfonylureas, biguanides, thiazolidinediones, and α-glycosidase inhibitors act to selectively modulate a specific pathological pathway (Rang and Dale, 1991 and Krentz and Bailey, 2005). As a result, these drugs control blood glucose levels provided they are regularly administered. Eventhough these drugs may be valuable in the management of diabetes mellitus, they have limitations due to undesirable adverse effects such as hypoglycemia, weight gain and inability to arrest pancreas degeneration (Harrower, 1994) or diabetic complications which have been linked to oxidative stress (Baynes, 1991).

Oxidative stress has a major role in development, progression, and complications of diabetes, therefore antioxidants may serve as a potential therapy for ameliorating the effect of oxidative stress (Maritim *et al.*, 2003). Thus, an ideal therapy for diabetes mellitus would be a drug that not only possesses antihyperglycemic effect, but also enhances or protects the antioxidant defense system which is usually compromised. Unfortunately, among the currently available hypoglycemic agents, the choice is very limited (Erejuwa *et al.*, 2011).

Aminoguanidine (AG) is one of many derivatives of guanidine (Nilsson, 1999) but it has many properties in common with hydrazines and is therefore often classified as a nucleophilic hydrazine compound (El-Khatib *et al.,* 2001). It is a prototype α, ß-dicarbonyl scavenging agent that prevents the formation of advanced glycation endproducts from α, ß-dicarbonyl precursors (Ihm *et al.,* 1999). Brodiak and Sybirna (2006) mentioned that AG is proposed to be used for pharmacological correction of nitric oxide (NO) biosynthesis, being the selective inducible nitric oxide synthase (iNOS) inhibitor, antioxidant, weakens the toxic effects of NO and positively modulates the pathological state caused by NO hyperproduction. Szabo *et al.* (1997) reported that AG has antioxidant and free radical scavenger properties especially on peroxynitrite ($ONOO^-$) production. Prasadachari and Vellaichamy (2011) confirmed the anti-hyperglycemic and anti-oxidative cardioprotective role of AG which could exert beneficial effects against diabetes and associated free radicals complications. Aminoguanidine protects STZ-induced renal pathological changes probably by attenuating diabetes-induced IL-1β, IL-6 and TNF-α gene expression (Vadla and Vellaichamy, 2012) and has a beneficial effects against histological injury induced by STZ by reducing the thickness of tunica media in diabetic aorta (Elbe et al., 2014).

Nicorandil is a nicotinamide derivative, efficacious in the treatment of hypertension and angina pectoris. It is a potassium channel opener providing vasodilatation of arterioles and large coronary arteries. Its nitrate component produces venous vasodilatation (Sweetman, 2005). Kasono *et al.,* (2004) found that nicorandil may possess anti-free radical characteristics, since the nicotinamide moiety of its molecular structure is a known hydroxyl radical scavenger. Moreover, this drug has been reported to show an inhibitory effect on superoxide anion production (Pieper and Gross, 1992 and Mano *et al.,* 2000). Serizawa *et al.,* (2011) investigated the beneficial effect of nicorandil on endothelial function in streptozotocin induced diabetic rats; it ameliorated endothelial dysfunction in STZ rats through an antioxidative effect exerted by normalizing p47 phox and eNOS uncoupling.

In the light of these considerations, this study was designed to evaluate the role of aminoguanidine and nicorandil and their combination against oxidative stress in diabetes.

2. MATERIALS AND METHODS

2.1.Marerials

Streptozotocin was obtained from Sigma, USA; Aminoguanidine was obtained from Merck, Germany; Nicorandil was obtained from Sigma, USA; Kits for determination of oxidative stress parameters: (SOD, catalase, MDA, GSH and NO) were obtained from Biodiagnostic company, Egypt; Kits for determination of blood glucose level were obtained from Vitro Scient, Egypt.

2.2. Animals

The study was conducted on 42 male Wistar albino rats, weighing 150-200g, were purchased from the animal house, Faculty of Medicine, Assiut University, Egypt and were housed in animal facility, Faculty of Medicine, Sohag University, maintained in a controlled environment under standard conditions of temperature (25±2ºC). A time controlled system provided 07.00–21.00 h light and 21.00–07.00 h dark cycles. All rats were given ad libitum access to rodent chow diet and water from sanitized bottle fitted with stopper and sipper tubes. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Sohag University, Faculty of Medicine.

2.3. Experimental protocol

2.3.1. Induction of diabetes in rats

After acclimatization for 1 week, animals were treated with a single dose of 1 ml of streptozotocin (STZ) 65 mg/kg dissolved in 0.1 mol/l sodium citrate at pH 4.5 intraperitoneally (Ihm *et al.,* 1999). Diabetes was considered to have been induced when the blood glucose level was higher than 250 mg/dl (Serizawa *et al.,* 2011) which was confirmed 48 hours after STZ administration by using drops of blood from retro-orbital plexus and measuring blood glucose level by using blood glucose kits.

2.4. Treatment

Control, non-diabetic and diabetic animals were used in the experiments. Control group (6 rats) was treated with pure vehicles.

Non-diabetic animals were divided into 2 groups 6 rats each: aminoguanidine treated group with 50 mg/kg intraperitoneally dissolved in saline (Chang *et al.,* 2006) and nicorandil treated group with 0.1 mg/kg orally by gastric tube dissolved in saline (Raveaud *et al.,* 2009).

Diabetic animals were divided into 4 groups 6 rats each: STZ- treated group, STZ+ aminoguanidine treated group (50 mg/kg aminoguanidine intraperitoneally dissolved in saline, 48 hours after STZ administration), STZ+ nicorandil treated group (0.1 mg/kg nicorandil orally by gastric tube dissolved in saline, 48 hours after STZ administration), and combination group, STZ+ aminoguanidine + nicorandil treated group (50 mg/kg aminoguanidine

intraperitoneally dissolved in saline and 0.1 mg/kg nicorandil orally by gastric tube dissolved in saline, 48 hours after STZ administration).

2.5. Sample collection

All of the above groups received their treatment for one month. At the end of the treatment period, all animals were sacrificed. Blood samples were obtained for measurement of blood glucose level and oxidative stress parameters (SOD, CAT, MDA, GSH and NO) by using commercially available kits, Egypt.

2.6. Histopathological examination

Samples of kidney and liver, from all of the above groups, were excised, fixed in 10% formaldehyde, and dehydrated in ascending grades of ethanol, cleaned in xylene and embedded in paraffin wax. Sections (5 µm thick) were cut and stained with hematoxylin and eosin (H&E).

2.7. Statistical analysis of data

Results were expressed as means ± standard error (SE) of the mean. The significance of difference between different groups was analyzed using one-way analysis of variance (ANOVA) followed by post hoc analysis with least significant difference (LSD) using SPSS program (version 9). The difference was regarded as significant when $p < 0.05$.

3. RESULTS

3.1. STZ- induced changes in rats

Treatment of the rats with 65 mg/kg STZ as a single intraperitoneal dose led to a significant increase in the plasma glucose level (Table 1) and significant reduction in plasma SOD, CAT and GSH levels compared to the control group (Figures 1, 2 and 4). It produced a significant increase in plasma MDA and plasma nitrite level compared to the control group (Figures 3 and 5).

Histopathologically, compared to control group, STZ produced accumulation of bile pigments inside the hepatocytes (cholestasis) and pyknotic nuclei with the presence of dilated congested portal vein in the liver (Figure 6A, B, C). In addition, congested venules with lymphocytic infiltrate, as well as hydropic degeneration of the tubular epithelial cells with focal interstitial hemorrhage were detected in the kidney (Figure 7A, B, C).

3.2. Effect of aminoguanidine, nicorandil and their combination on STZ- induced changes

3.2.1. Plasma glucose level

Treatment of the diabetic rats with either 50 mg/kg aminoguanidine intraperitoneally, 0.1mg/Kg nicorandil orally or a combination of aminoguanidine and nicorandil produced insignificant effect on the plasma glucose level compared to STZ- treated group (Table 1).

3.2.2. Oxidative stress parameters

Administration of 50 mg/kg aminoguanidine intraperitoneally to the diabetic rats produced significant increase in plasma SOD, CAT and GSH levels (Figures 1, 2 and 4), and significant decrease in plasma MDA and plasma nitrite levels compared to STZ- treated group (Figures 3 and 5).

Administration of 0.1 mg/kg nicorandil orally to the diabetic rats produced a significant increase in plasma SOD and GSH levels (Figures 1 and 4) and significant decrease in plasma MDA level compared to STZ- treated group (Figure 3). It produced insignificant change in plasma CAT and nitrite level compared to STZ- treated group (Figures 2 and 5).

Treatment of the diabetic rats with combination of 50 mg/kg aminoguanidine intraperitoneally and 0.1 mg/kg nicorandil orally led to significant increase in plasma SOD, CAT and GSH levels (Figures 1, 2 and 4) and significant decrease in plasma MDA and plasma nitrite levels compared to STZ- treated group (Figures 3 and 5).

This combination produced insignificant change in plasma SOD, CAT, GSH and NO levels compared to STZ + aminoguanidine group (Figures 1, 2, 4 and 5), but significantly decreased plasma MDA level (Figure 3).

Compared to STZ + nicorandil group, this combination led to significant increase in plasma CAT and GSH levels (Figures 2 and 4) and significant decrease in plasma MDA and nitrite levels (Figures 3 and 5) but produced insignificant change in plasma SOD level (Figure 1).

3.2.3. Histopathological examination of liver and kidney

Liver of diabetic rats treated with aminoguanidine showed only mild focal hydropic degeneration of hepatocytes (Figure 6D). Liver of diabetic rats treated with nicorandil showed no significant pathological changes except few dilated veins (Figure 6F). While, liver of diabetic rats treated with combination aminoguanidine + nicorandil showed mild focal hydropic degeneration of the hepatocytes (Figure 6H).

Kidney of diabetic rats treated with aminoguanidine showed no significant pathological changes (Figure 7D). Kidney of diabetic rats treated with nicorandil showed only mild focal interstitial hemorrhage (Figure 7F). Furthermore, kidney of

diabetic rats treated with combination of aminoguanidine + nicorandil showed only mild focal hydropic degeneration of the tubular epithelial cells (Figure 7H).

3.3. Effect of aminoguanidine, nicorandil in non-diabetic rats

3.3.1. Plasma glucose levels

Treatment of the non-diabetic rats with either 50 mg/kg aminoguanidine intraperitoneally or 0.1 mg/kg nicorandil orally produced insignificant effect on the plasma glucose level compared to control group (Table 1).

3.3.2. Oxidative stress parameters

Treatment of the non-diabetic rats with either aminoguanidine or nicorandil produced insignificant effect on plasma SOD, CAT, MDA, GSH and NO levels compared to control group (Figures 1, 2, 3, 4 and 5).

3.3.3. Histopathological examination of kidney and liver

Liver of non-diabetic rats treated with either aminoguanidine or nicorandil showed no significant pathological changes (Figure 6E, G).

Kidney of non-diabetic rats treated with either aminoguanidine or nicorandil showed no significant pathological changes (Figure 7E, G).

Table 1: Effect of treatment with 50 mg/kg aminoguanidine intraperitoneally, 0.1mg/kg nicorandil orally and their combination on plasma glucose level in rats given streptozotocin (STZ) 65 mg/kg intraperitoneally.

Groups	Plasma glucose level (mg/dl)
Control	95.67 ± 1.43
Aminoguanidine	97.00 ± 1.71
Nicorandil	96.17 ± 1.66
STZ	354.67 ± 8.89*
STZ + aminoguanidine	333.95 ± 5.23*
STZ + nicorandil	343.00 ± 6.88*
STZ + aminoguanidine +nicorandil	350.50 ± 7.03*

Each value represents the mean ± SEM of 6 rats. Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).
* Significant difference at P<0.05 Vs. control values.

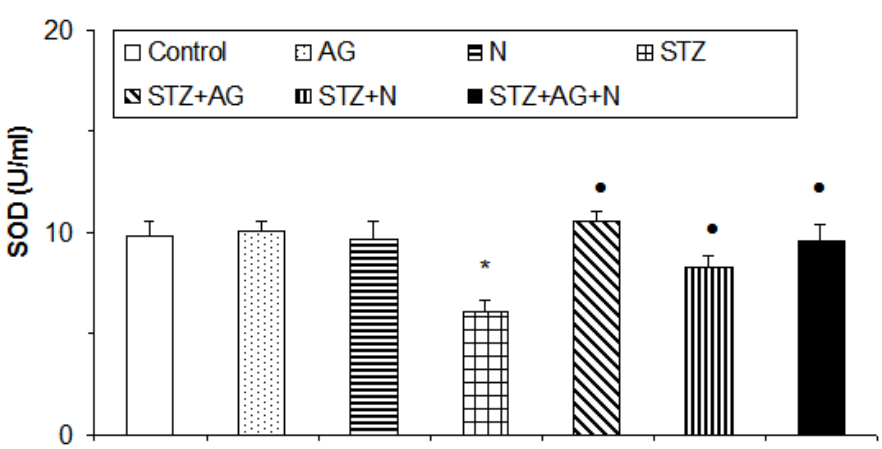

Figure 1: Effect of aminoguanidine 50mg/kg intraperitoneally and nicorandil 0.1mg/kg orally and their combination on streptozotocin-induced disturbances in plasma SOD level.
AG= aminoguanidine, N= nicorandil and STZ= streptozotocin.
Results are presented as mean ± SEM of 6 rats. Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).
* Significant difference at P<0.05 Vs control group.
• Significant difference at P<0.05 Vs STZ- treated group.

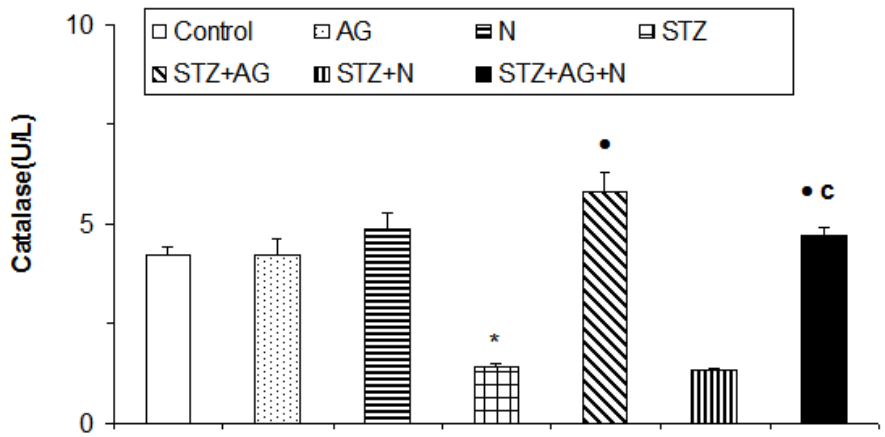

Figure 2: Effect of aminoguanidine 50 mg/kg intraperitoneally and nicorandil 0.1 mg/kg orally and their combination on streptozotocin induced disturbance in plasma catalase level. AG= aminoguanidine, N= nicorandil and STZ= streptozotocin.

Results are presented as mean ± SEM of 6 rats. Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).

* Significant difference at P<0.05 Vs control group.

• Significant difference at P<0.05 Vs STZ- treated group.

[c] Sgnificant difference at P<0.05 Vs STZ+N treated group.

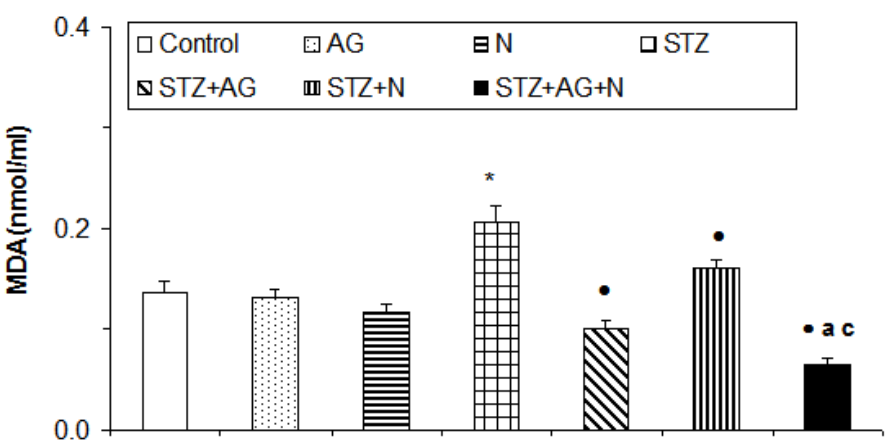

Figure 3: Effect of aminoguanidine 50 mg/kg intraperitoneally and nicorandil 0.1 mg/kg orally and their combination on streptozotocin-induced disturbances in plasma MDA level.

AG= aminoguanidine, N= nicorandil and STZ= streptozotocin.

- Results are presented as mean ± SEM of 6 rats. Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).

* Significant difference at P<0.05 Vs control group.

• Significant difference at P<0.05 Vs STZ- treated group.

[a] Significant difference at P<0.05 Vs STZ+AG treated group.

[c] Significant difference at P<0.05 Vs STZ+N treated group.

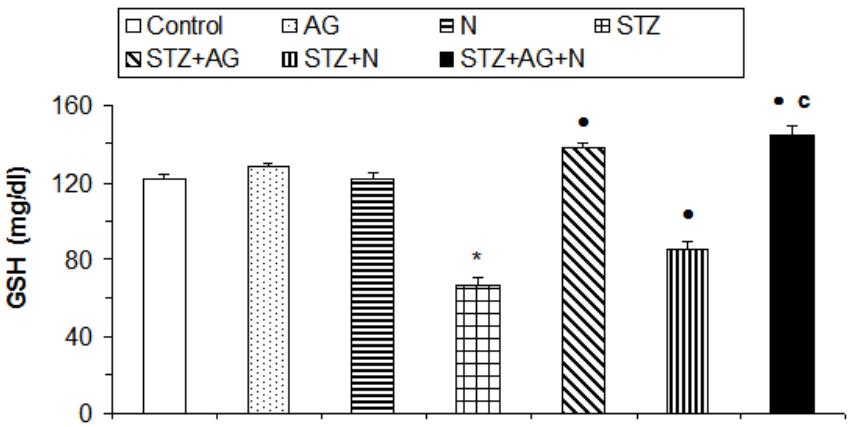

Figure 4: Effect of aminoguanidine 50 mg/kg intraperitoneally and nicorandil 0.1 mg/kg orally and their combination on streptozotocin-induced disturbances in plasma GSH level.
AG= aminoguanidine, N= nicorandil and STZ= streptozotocin.
Results are presented as mean ± SEM of 6 rats. Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).
* Significant difference at P<0.05 Vs control group.
• Significant difference at P<0.05 Vs STZ- treated group.
[c] Significant difference at P<0.05 Vs STZ+N treated group.

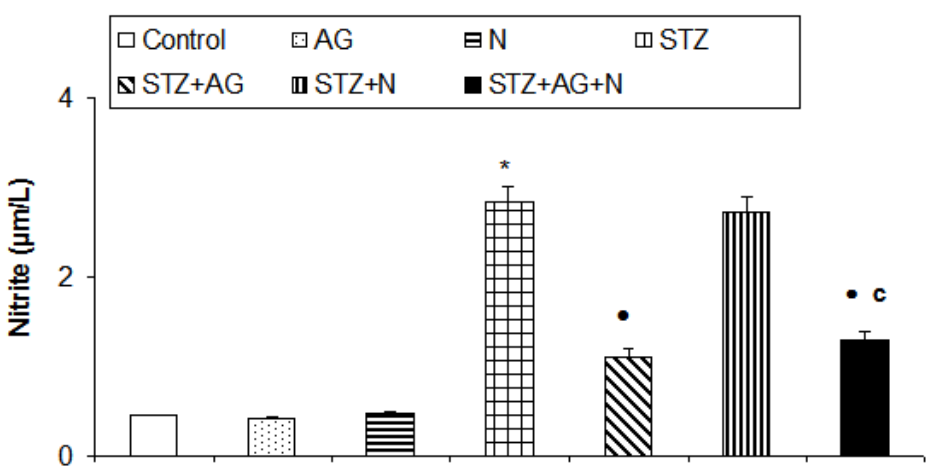

Figure 5: Effect of aminoguanidine 50 mg/kg intraperitoneally and nicorandil 0.1 mg/kg orally and their combination on streptozotocin-induced disturbances in plasma nitrite level.
AG= aminoguanidine, N= nicorandil and STZ= streptozotocin.
Results are presented as mean ± SEM of 6 rats. Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).
* Significant difference at P<0.05 Vs control group.
• Significant difference at P<0.05 Vs STZ- treated group.
[c] Significant difference at P<0.05 Vs STZ+N treated group.

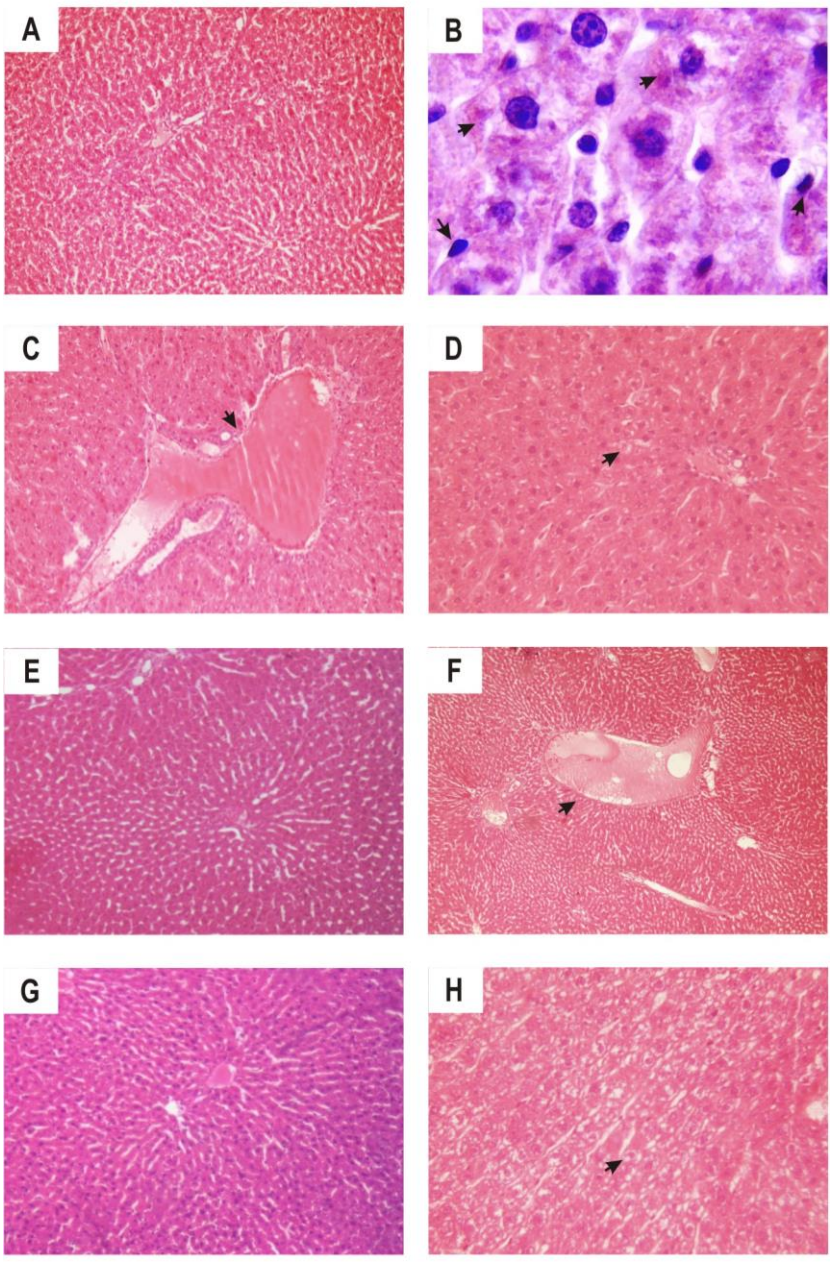

Figure 6: Photomicrographs of the liver tissue showing effects of aminoguanidine, nicorandil and their combination on streptozotocin– induced diabetes in rats.

A: Liver of control group showing normal tissue structure (H&E X100).

B: Liver of STZ- treated group showing accumulation of bile pigments inside the hepatocytes (cholestasis**)** and pyknotic nuclei (H&E X400).

C: Liver of STZ- treated group showing a dilated congested portal vein (H&E X100).

D: Liver of treated diabetic group with aminoguanidine showing mild focal hydropic degeneration of the hepatocytes (H&E X200).

E: Liver of non diabetic rats treated with aminoguanidine showing no significant pathological changes (H&E X100).

F: Liver of treated diabetic group with nicorandil showing no significant pathological changes except few dilated veins (H&E X100).

G: Liver of non diabetic rats treated with nicorandil showing no significant pathological changes (H&E X100).

H: Liver of treated diabetic group with combination of aminoguanidine and nicorandil showing mild focal hydropic degeneration of the hepatocytes (H&E X200).

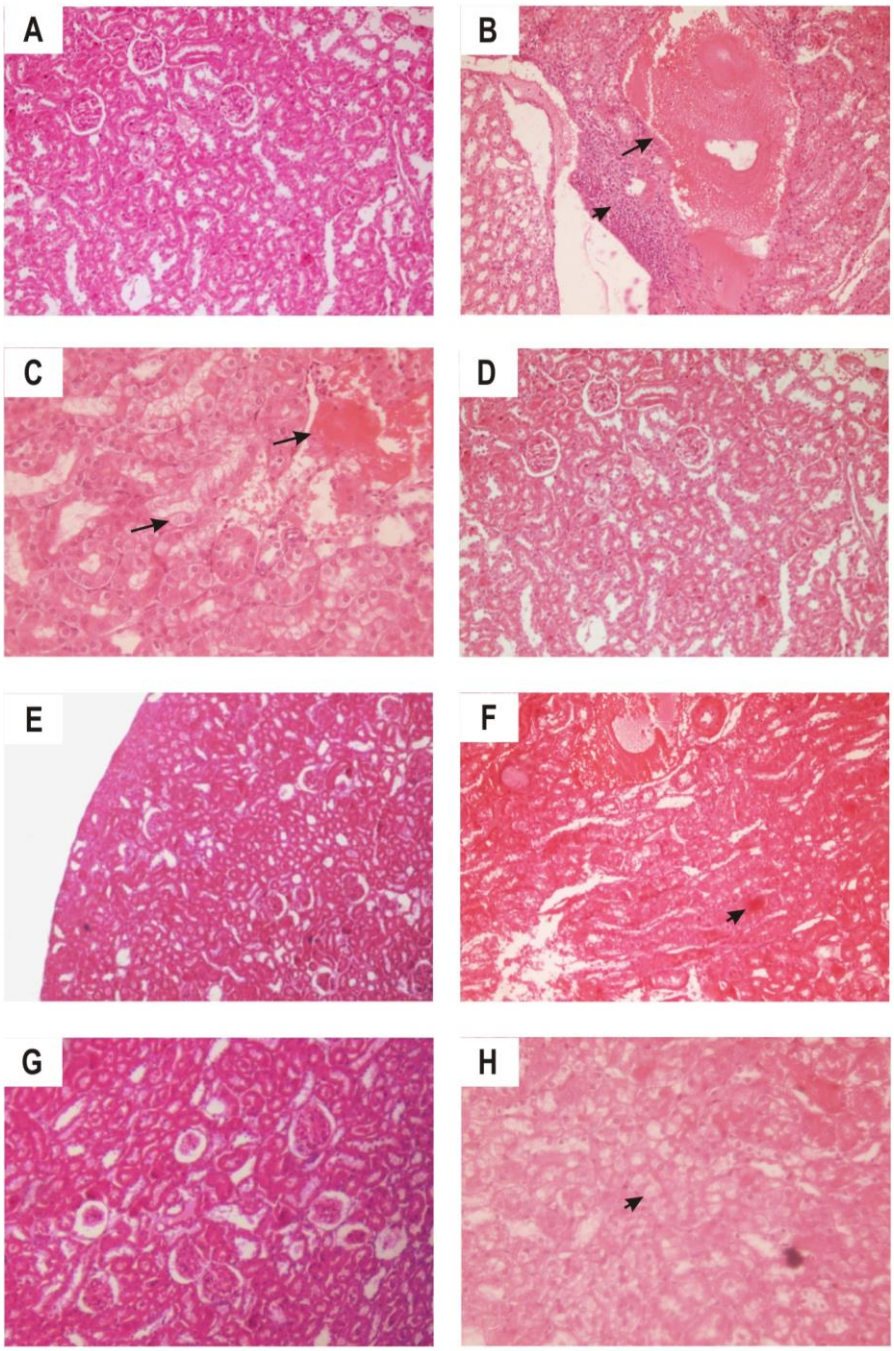

Figure 7: Photomicrographs of the kidney tissue showing effects of aminoguanidine, nicorandil and their combination on streptozotocin– induced diabetes in rats.

A: Kidney of control group showing normal tissue structure (H&E X200).
B: Kidney of STZ- treated group showing congested venules with lymphocytic infiltration (H&E X100).
C: Kidney of STZ- treated group showing hydropic degeneration of tubular epithelial cells with focal interstitial hemorrhage (H&E X200).
D: Kidney of treated diabetic group with aminoguanidine showing no significant pathological changes (H&E X200).
E: Kidney of non diabetic rats treated with aminoguanidine showing no significant pathological changes (H&E X200).
F: Kidney of treated diabetic group with nicorandil showing focal interstitial hemorrhage (H&E X100).
G: Kidney of non diabetic rats treated with nicorandil showing no significant pathological changes (H&E X200).
H: Kidney of treated diabetic group with combination of aminoguanidine and nicorandil showing mild focal hydropic degeneration of the tubular epithelial cells (H&E X200).

4. DISCUSSION

Diabetes mellitus evolved as a major threat to human beings worldwide. It is characterized by a lack of insulin causing elevated blood glucose, often with associated insulin resistance (Tripathi and Srivastava, 2006).

Diabetes and its complications are related to multiple pathogenic factors including hyperglycemia and oxidative stress. Oxidative stress plays key role in mediating pathogenesis of diabetes and its complications (Prasadachari and Vellaichamy, 2011).

Hyperglycemia causes increased protein glycation, which has been known to be a source of free radicals. Reactive oxygen species and the products of advanced glycation are significant in the development of complications in the chronic diabetes (Basta *et al.,* 2004 and Maiese *et al.,* 2007).

So, in this study we investigated and compared the effect of an antioxidant; aminoguanidine and nicorandil and their combination in STZ-induced diabetic rats.

Streptozotocin induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia (Low *et al.,* 1997). At the beginning of diabetes, inflammation of pancreas islet is frequently observed and free radicals released from phagocytes play important role in this inflammation (Celik and Akkaya, 2009).

In the present study, intraperitoneal injection STZ produced reduction in plasma SOD, CAT and GSH levels and elevation in plasma MDA and NO levels, there was also an elevation in plasma glucose level. Our results are in harmony with the work of Grover *et al.,* (2000), Daisy *et al.,* (2010) and Guimaraes *et al.,* (2011). They reported that the increase in blood glucose level depends upon the degree of pancreatic β-cell destruction. The diabetogenic agent STZ selectively destructs β-cells of the islets of Langerhans in the pancreas result in inhibition of insulin synthesis and elevation of blood glucose level.

The biochemical changes as elevation of blood glucose level, MDA and NO and reduction of anti-oxidant parameters (SOD, catalase and GSH) are evidences of STZ-induced hyperglycaemia in rats (Young *et al.,* 1995; Ihm *et al.,* 1999; Coskun *et al.,* 2005; El- Sayed *et al.,* 2009 and Prasadachari and Vellaichamy, 2011).

The main histopathological changes detected in kidney tissue obtained from the rats injected with STZ were congested venules with lymphocytic infiltration and hydropic degeneration of tubular epithelial cells with focal interstitial hemorrhage. Accumulation of bile pigments inside the hepatocytes (cholestasis), dilated congested portal vein in the liver specimens obtained from the same animals. Our findings agree with de Cavanagh *et al.,* (2001) and Kushwaha *et al.,* (2011).

Aminogunidine (AG) demonstrated the capacity to inhibit the formation of advanced glycation endproducts. Besides, being a NOS inhibitor, it has been described as a protective agent in several diabetic complications such as neuropathy, nephropathy, ocular changes, cardiomyopathy and vascular dysfunctions (Kern and Engerman, 2001).

In the current study, treatment of hyperglycemic animals with AG significantly increased the antioxidant enzymes levels (SOD and Catalase) and GSH level and significantly decreased MDA and NO levels. Also it produced insignificant effect on the elevated plasma glucose level as compared to hyperglycaemic non-treated rats. Our observation is in agreement with other reports (El-Khatib *et al.,* 2001). showing the beneficial effects of AG against many diabetic complications induced by STZ despite the presence of severe unaltered hyperglycaemia.

As previously mentioned AG is a well-known selective inhibitor of inducible nitric oxide synthase. The reaction between NO produced by endothelial cells and superoxide anion yields peroxynitrite, which is itself a powerful oxidizing agent capable of initiating lipid peroxidation. In diabetes, increased glucose and free fatty acid concentrations may stimulate endothelial cells and macrophages to secrete NO and superoxide, resulting in increased peroxynitrite formation and increased lipid peroxidation (Ihm *et al.,* 1999). Thus, one cannot exclude the possibility that inhibition of NO production by AG observed in this study may have contributed to the inhibition of lipid peroxidation.

Mustafa *et al.* (2002) mentioned that AG directly scavenges hydroxyl radicals and thereby inhibits lipid peroxidation. Reduction in MDA levels in the AG-treated rats is probably due to AG antioxidant and free radical scavenging properties (Ara *et al.,* 2006).

Prasadachari and Vellaichamy (2011) reported that AG exhibits antiperoxidative and antioxidant activities in heart tissue of streptozotocin induced diabetic mice by decreasing the levels of lipid peroxidation products and increasing the levels and activities of antioxidants. Thus, the role of AG in management of diabetes is of paramount importance

and may serve various purposes in diabetics and delay complications by reducing free radical induced tissue damage.

Nicorandil is an orally available drug that can act as a nitric oxide donor, an antioxidant, and an ATP-dependent K^+ channel opener, and has a beneficial role in treating diabetic nephropathy (Tanabe *et al.,* 2012). A pure ATP-dependent K^+ channel opener did not inhibit lipid peroxidation, suggesting that the anti-oxidative action of nicorandil is not mediated via a K^+ channel (Naito *et al.,* 1994). It may also act as an antioxidant via the formation of nitric oxide, which can interfere with free radical production (Pieper *et al.,* 1994)

In our study treatment of diabetic rats with nicorandil lead to significant increase SOD and GSH levels and significant decrease in MDA level but produced insignificant effects on both plasma glucose level, catalase and NO levels, compared to the STZ-treated groups.

Mano *et al.* (2000) reported that increased lipid peroxidation in the serum, kidneys, and cardiac muscle of diabetic rats was restored to the same level as the control rats by nicorandil treatment. Thus, nicorandil may protect various organs against lipid peroxidation invasion under diabetic conditions. The decreases in free radical scavenging enzyme levels in the diabetic state were improved by nicorandil treatment (Naito *et al.,* 1994). Probably, the high levels of lipid peroxidation induced by hyperglycemia resulted in the induction of scavenging enzymes, and these distorted metabolic changes were recovered by nicorandil treatment. Serizawa *et al.* (2011) reported that nicorandil could inhibit the expression and activity of NADPH oxidase, a major source of ROS (Muller and Morawietz, 2009), leading to the reduction of ROS production. Tanabe *et al.* (2012) reported that nicorandil had no effect on blood glucose level in STZ-induced diabetic mice. Nicorandil appeared to have a protective effect on the lipid peroxidation, as evidenced by scavenging free radical and increasing the antioxidant enzyme activity (Taye *et al.,* 2008).

In our study, combined treatment with aminoguanidine and nicorandil to diabetic animals corrected the abnormalities produced by STZ in all the studied parameter. This result suggests the possible antioxidant activity of the combination. The result showed that the previous combination produced no effect on the elevated plasma glucose level as compared to STZ- treated rats. Moreover, the result showed that, combined treatment with aminoguanidine and nicorandil to the diabetic rats had no effect in SOD, catalase, GSH and NO levels but decreased MDA level compared to the diabetic groups treated with aminoguanidine alone. However, this combination increase catalase and GSH levels, and decrease MDA and NO levels and produced no effect in SOD level compared to the diabetic groups treated with nicorandil alone.

This study showed a beneficial effect of aminoguanidine, nicorandil and their combination on the tissue structures of the kidney and liver, there is an inverse relationships observed between tissue changes produced by STZ administration and anti-oxidant levels. Streptozotocin produced decrease in SOD, catalase and GSH levels and increase in MDA and NO levels and produced abnormalities in both kidney and liver structures. Administration of either AG, nicorandil or their combination produced increase in SOD, catalase and GSH levels and decrease in MDA and NO levels and improvement of the histopathological abnormalities produced by STZ.

In conclusion, the results suggest that aminoguanidine, nicorandil and their combination have the ability to improve oxidative stress induced by streptozotocin. The ability of aminoguanidine, nicorandil and their combination to provide this effect is positively correlated with their anti-oxidant activities, by increasing the level of SOD, catalase and GSH, decreasing the level of MDA and NO and correcting the pathological abnormalities produced by STZ in the kidney and liver tissues. Thus, the anti-oxidant activities of aminoguanidine, nicorandil and their combination may play a pivotal role in protection against oxidative stress in STZ- induced diabetes mellitus.

6. ACKNOWLEDGEMENT

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